

Influence of alternative plant nutrition methods on soil microbial characteristics in long-term experiments

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Abstract

The size of the arable land is constantly decreasing all over the world due to severe anthropogenic disorders. Plant production therefore has to be adapted to changing environmental conditions along with the proper selection of crop varieties and the application of sustainable environmental technologies which also consider economic aspects. The investigations were carried out in the Westsik long-term fertilization experiment near Nyíregyháza, East Hungary, which was set up in 1929 (89 years ago). Alternative forms of nutrient supplies (A) (green manure, straw with and without fermentation, organic fertilizer with and without inorganic fertilizer supplements) were used in different crop rotations. The test plant was potato (*Solanum tuberosum* L.) and the soil type sand with a low humus content (Arenosols). A further long-term experiment is located on calcareous chernozem soil (Chernozems) in Debrecen (set up in 1983, 35 years ago). In one part of this experiment, organic farming (OF) has been carried out with a pea, winter wheat and maize crop rotation for over 15 years with no inorganic fertilization. In another block in this experiment, changes in soil properties as a result of the medium and high doses of fertilizers applied in intensive farming (I) were evaluated with a maize (*Zea mays* L.) monoculture as the test plant.

The results obtained with alternative nutrient supplies (green manure, fermented and unfermented straw, farmyard manure, fertilization) proved that the soil organic carbon content increased to varying degrees in humus-poor, acidic sand soil. The organic matter content of the soils increased in response to the treatments, contributing to a significant enhancement in soil microbial parameters (MBC, saccharase, dehydrogenase and phosphatase enzyme activities).

The carbon dioxide production and saccharase enzyme activity in organic plots (OF) were significantly lower than in intensively farmed (I) soils. At the same time, in the case of organic farming (OF) the microbial biomass carbon, phosphatase and dehydrogenase activity were significantly higher in OF plots than in I plots.

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Compared to the control soil, MBC was 7-8 times higher in organic plots and 1.3-3.8 times higher in intensive plots.

Organic farming on chernozem soil generally resulted in higher microbial activity (MBC, phosphatase, saccharase and dehydrogenase enzyme activity) than in either intensively farmed chernozem or in the case of alternative farming (A) on sandy soil.

Introduction

The aim of crop production all over the world is to maintain or increase crop yields in order to produce larger quantities of better quality food and feed raw materials. Crop production has to adapt to changing environmental conditions through the selection of varieties and the application of environmentally sound, economical technologies (LOCH and NOSTICZIUS 2004).

Among the ecological factors, the physical, chemical and biological properties of the soils are of paramount importance, so the complex knowledge of these properties is essential (KÁTAI, 1992). Without this knowledge it is impossible to achieve rational soil use, sustainable soil fertility, and profitable farming (BÍRÓ, 2005).

However, the direction and intensity of the transformation of organic matter depends not only on the soil humus content and the organisms living in the soil, but also on the natural fertility of the soil, the effects of environmental factors and the technology applied. The intensity and speed of humification and mineralization determine the amounts of both organic matter and plant-available minerals in the soil (FÜLEKY and RAJKAINÉ, 1999).

Various organic substances and fertilizers (plant residues, manure, biogas fermentation residues, green manure, compost and slurry) generally have favourable effects on soil biodynamics (MÜLLER, 1991; KÁTAI et al., 1999; KÁTAI 2000; SZILI-KOVÁCS et al., 2009), providing direct nutrient sources for saprotrophic microbes. Other positive effects are caused by humification, better soil structure and improved air and water management.

The effects of fertilizer application on the chemical and microbiological properties of soils have been investigated in many long-term experiments (JANUSAUSKAITE et al., 2013; GEISSELER and SCOW, 2014; KÁTAI et al., 1999, 2014; SZILI-KOVÁCS et al., 2009; POWLSON and JOHNSTON, 1994; SIMON and CZAKO, 2014; ZHAO et al., 2013), which demonstrated that a balanced nutrient supply has a positive effect on the nutrient cycles, energy flow, biodynamics and activity of soil organisms, and thus on the growth and development of plants.

The requirements of organic, integrated and intensive farming vary considerably. Organic farming is based on organic fertilization and biological plant protection, without the use of artificial fertilizer or dangerous plant protection agents (SCHRAMA et al., 2018). The aim of integrated management is to optimize soil fertility and plant nutrition to achieve the desired crop yield (SÁRDI, 2011). The purpose of intensive farming is to achieve the greatest yield. Different plant

nutrition methods have many direct and/or indirect effects on soil conditions, on nutrient reserves and nutrient content, and on microbiological processes.

The purpose of this study was to compare different forms of nutrient supplies (green manure, livestock manure, inorganic fertilizers and various combinations of these) in organic and conventional farming systems. It was hypothesised that i) organic farming increases soil microbiological biomass and activity compared to conventional farming; ii) there is a close connection between soil nutrient status and microbial biomass and activity, and that this correlation is determined by the farming system; iii) the soil type has a defining role in the intensity of microbial processes. To prove this hypothesis, investigations were made on chemical soil properties and on microbial biomass and activity.

Materials and methods

Experimental area

Experiment I.

One part of the investigations was carried out in a long-term fertilization experiment, set up in 1929 by Vilmos Westsik near Nyíregyháza, in the eastern part of Hungary. In this experiment various forms of nutrient supply (green manure, fermented or non-fermented straw, animal manure) were used with or without inorganic fertilizers in different crop rotations on sand with low humus content (WRB: Arenosols) and acidic pH. The test plant was potato. Six of the 15 treatments were selected for the present experiment:

- I. AC: control, fallow, without cultivation or fertilization
- II. AGM-N: with main-crop lupine as green manure, combined with N fertilizer
- IV. AS-NPK: with fermented straw and NPK fertilizer
- VII. AS: with straw but no inorganic fertilizers
- XI. AF-N: with farmyard manure and N fertilizer
- XIV. AGM-PK: with second-crop lupine as green manure, ploughed in soil, in autumn, and PK fertilizer

Experiment II.

In another long-term fertilization experiment, set up in 1983 on a highly fertile calcareous chernozem soil (WRB: Chernozems) in the Hajdúság loess area west of Debrecen in East Hungary, organic farming has been carried out in one block for more than 15 years, with no inorganic fertilizer and a constant maize-wheat-pea crop rotation. The other block involved intensive farm management with medium and high doses of fertilizer. The test crop here was a maize monoculture.

Treatments:

1. Control (C) (maize, without fertilization)

Organic farming (OF)

2. OF, P (pea)
3. OF, M (maize)

Intensive farm management (using solid NH_4NO_3 , $\text{NH}_4\text{H}_2\text{PO}_4$ and KCl fertilizers)

4. Medium dose of fertilizers (IMF1): 120, 90, 90 kg ha⁻¹ N, P₂O₅, K₂O, resp.

5. Large dose of fertilizers (IMF2): 240, 180, 180 kg ha⁻¹ N, P₂O₅, K₂O, resp.

Soil samples were taken from 5 points/treatments at a depth of 2-20 cm on 10 May 2016.

Chemical and biological soil properties investigated

Laboratory analyses on moisture content (105°C), pH (BUZÁS, 1988), organic carbon content (OC) (SZÉKELY et al., 1960), soil respiration (CO₂ production) (ÖHLINGER, 1996), microbial biomass carbon (MBC) (VANCE et al, 1987), dehydrogenase activity (MERSHI, 1996), phosphatase activity (SZEGI, 1979) and saccharase activity (FRANKENBERGER and JOHANSON, 1983) were carried out in four replications.

Table 1

Effect of alternative management (A) on some physical and chemical parameters of humiferous sandy soil (Arenosol) (Nyíregyháza, May 2016); test plant: potato

Treatment code	Moisture content %	pH in distilled water	pH in 1.0 M KCl solution	Humus content %	Organic C content g kg ⁻¹
I. Control AC	5.49	5.40	3.88	0.51a	3.0a
II. AGM-N	5.97	4.76	3.51	0.54a	3.1a
IV. AS-NPK	5.66	4.37	3.51	0.62ab	3.6ab
VII. AS	6.58	6.24	4.93	0.64ab	3.7ab
XI. AF-N	5.64	5.37	4.44	0.83c	4.8c
XIV. AGM-PK	7.58	5.18	3.74	0.83c	4.8c
<i>LSD</i> _{5%}	-	-	-	0.09	0.5

For treatment details, see Materials and methods

Statistical analyses

One-way ANOVA was used for determining treatment effects and the standard deviation (S.D.) of the means was calculated using the SPSS 13.0 program at the 5.0 % significance level. After ANOVA, Duncan's multiple range test was used to compare the means. Values indicated with different letters were significantly different from each other at the 5.0 % significance level. Pearson correlation analysis was used to reveal correlations between microbiological and chemical soil properties.

Results and discussion

Westsik research experiment (Experiment I)

The soils in the Westsik experiment in Nyíregyháza had very low moisture content at the sampling date (*Table 1*). Based on the pH values, the soils could be classified as acidic, with the exception of the AS-F treatment, which was only slightly acidic. The soils also had low or very low organic carbon content, which rose slightly compared to the control in response to green manure (AGM-N), straw

(AS) and straw + nitrogen fertilizer (AS-N), and increased significantly in the AF-N and AGM-PK treatments.

The carbon dioxide production of the soils increased slightly in all the treatments (*Table 2*), but only showed a significant increase in the AF-N treatment. Microbial biomass carbon (MBC) significantly increased in all the treatments, with particularly high values in the green manure treatments (AGM-N, AGM-PK). The humiferous sandy soil had very low saccharase enzyme activity in the control plot, but the values were more than doubled by the straw (AS-NPK) and green manure plus PK fertilizer (AGM-PK) treatments. The highest phosphatase activity was found in the green manure – mineral fertilizer (AGM-PK) combination, followed by the farmyard manure plus mineral N fertilizer (AF-N) treatment. All treatments with the exception of unfermented straw (AS-NPK) resulted in significant increases (1.1 to 1.8 fold). Similarly high activity was measured for dehydrogenase in the AGM-PK and AF-N treatments, but except for the AGM-N treatment, a significant increase was observed in all the treatments compared with the control.

Table 2

Effect of alternative management on some soil microbial characteristics of humiferous sand (Arenosols) (Nyíregyháza, May 2016)

Treatment	CO ₂ mg/100g soil/10 days	MBC µg g ⁻¹	Saccharase glycose mg 100g ⁻¹	Phosphatase mg 100g ⁻¹ 2h ⁻¹	Dehydroge nase INTF µg 100g ⁻¹
I. Control AC	8.65a	134.21a	2.12a	11.23a	35.13a
II. AGM-N	8.89a	251.14b	2.52a	15.37b	37.73a
IV. AS-NPK	8.90a	192.89c	4.76b	12.21a	39.80b
VII. AS	8.87a	226.56d	3.97c	15.93b	40.15b
XI. AF-N	9.59b	163.17e	3.86c	17.13b	47.43c
XIV. AGM-PK	8.85a	256.62b	4.97b	20.22c	48.40c
LSD _{5%}	0.62	23.94	0.62	1.29	3.65

For treatment details, see Materials and methods

INTF = reduced iodonitrotetrazolium formazan

Debrecen-Látókép experimental site (Experiment II.)

The soils in the Debrecen-Látókép experiment had higher than average moisture content at the time of sampling (*Table 3*). The soil $pH_{(H_2O)}$ ranged from 5.93 to 6.27, and could be classified as slightly acidic. Compared to the untreated control, the soils of both organic (OF) and intensive farming (I) were more acidic. Compared with the control, the fertilizer treatments (IMF1, IMF2) used in intensive farming (I) caused a significant increase in the organic carbon content. On the organic plots (OF) the soil organic carbon content was lower than in the intensive plots, but did not differ significantly from the control.

Table 3

Effect of organic farming (OF) and intensive management (I) on some physical and chemical parameters of calcareous chernozem soil (Chernozems) (Debrecen-Látókép, May 2016)

Treatment	Crop	Moisture content %	pH in distilled water	pH in 1.0 M KCl solution	Humus content %	Organic C content g kg ⁻¹
a. Control MC	Maize	19.30	6.27	5.09	2.76a	16.0a
Organic farming						
b. OF Pea	Pea	20.56	5.99	4.88	2.62a	15.2a
c. OF Maize	Maize	22.43	5.93	5.12	2.75a	15.9a
Intensive farming						
d. Medium dose IMF1	Maize	19.19	6.04	4.85	3.03a	17.4b
e. Large dose IMF2	Maize	19.15	5.95	4.71	3.17ab	18.2b
LSD _{5%}		-	-	-	0.31	0.82

For treatment details, see Materials and methods

Table 4

Effect of organic farming (OF) and intensive management (I) on some microbial parameters of calcareous chernozem soil (Chernozems) (Debrecen-Látókép, May 2016)

Treatment	CO ₂ mg 100g soil ⁻¹ 10 days ⁻¹	MBC µg g ⁻¹	Saccharase glycose mg 100g ⁻¹	Phosphatase mg 100g ⁻¹ 2h ⁻¹	Dehydrogenase INTF µg 100g ⁻¹
a. Control MC	19.53a	36.95a	14.43a	12.75a	25.90a
Organic farming					
b. OF Pea	10.71b	245.50b	10.55b	20.47b	50.58b
c. OF Maize	10.76b	275.55b	9.39b	21.44b	53.10b
Intensive farming					
d. Medium dose IMF1	19.88a	51.37a	13.13a	13.67a	33.20c
e. Larger dose IMF2	19.98a	143.33c	13.16a	15.52a	33.75c
LSD _{5%}	1.07	34.30	1.65	3.85	3.85

INTF = reduced idonitrotetrazolium formazan

The carbon dioxide production and saccharase enzyme activity of the soil were significantly lower in the case of organic farming (OF) than for intensive farming (I) (Table 4). At the same time, the microbial biomass carbon, phosphatase and dehydrogenase activity were significantly higher on organic (OF) than on intensive plots (I). The highest phosphatase and dehydrogenase activity was measured in the organically farmed soils. Compared to the control soil, *MBC* was 7-8 times higher in the case of organic farm management (OF) and 1.3-3.8 times higher for intensive farming (I). Inorganic fertilizers (IMF1, IMF2) significantly increased the *MBC* values in the soil.

In intensive farming (I), the carbon dioxide production, saccharase and phosphatase enzyme activity were similar to the control values. Mineral fertilization (IMF1, IMF2) resulted in higher values for both enzymes compared to the control, but this increase was only slight for phosphatase. The dehydrogenase enzyme activity showed a significant increase in these treatments.

Table 5

Pearson's correlations between soil properties Management system A (Nyíregyháza): n=24;
Management systems OF and I (Debrecen-Látókép): n=20

Measured parameters	Management system	Moisture content	pH _(H₂O)	Organic carbon	CO ₂ production	MBC	S-ase activity	P-ase activity
a. Moisture content	A OF, I							
b. pH _(H₂O)	A OF, I	--						
c. Organic carbon	A OF, I	- -	- -					
d. CO ₂ production	A OF, I	- -	- -	- 0.775				
e. MBC	A OF, I	0.666 0.698	- 0.699	- -	- -0.885			
f. Saccharase activity	A OF, I	- -0.751	- 0.572	0.603 -	- 0.830	-0.822		
g. Phosphatase activity	A OF, I	0.684 0.591	- -0.58	0.728	- -0.824	0.615 0.843	- 0.757	
h. Dehydrogenase activity	A OF, I	- 0.635	- 0.713	0.871 -	- -0.874	- 0.885	0.620 - 0.830	0.757 0.832

All the given correlations are significant at the 0.01 level (2-tailed).

Management systems: organic (OF), intensive (I) and alternative (A) farming

MBC=microbial biomass carbon, S-ase= saccharase, P-ase=phosphatase

Correlations between physico-chemical and biological parameters

Pearson's correlations were calculated to reveal correlations between the chemical and biological properties of the soil (Table 5), and the correlations found between microbial biomass carbon and phosphatase enzyme activity, and between phosphatase and saccharase enzyme activity are depicted in Figures 1 and 2, respectively, from which it can be seen that these pairs of parameters were in close connection with each other.

Studies in the Westsik long-term experiment (KÁTAI et al., 1999) showed that the use of white lupine straw or green manure supplemented with NPK enhanced the nitrate, AL-soluble phosphorus and potassium contents of the soil. The addition of straw, green manure or livestock manure had a stimulating effect on the activity of the saccharase and phosphatase enzymes to varying degrees.

Among the microbiological properties of the soil, the value of MBC and the activity of the saccharase, urease and dehydrogenase enzymes were significantly increased by alternative nutrient supplies (green manure, farmyard manure and straw) in the acidic, humus-poor sandy soil tested in the Westsik experiment. These results were consistent with previous results (KÁTAI et al., 2002).

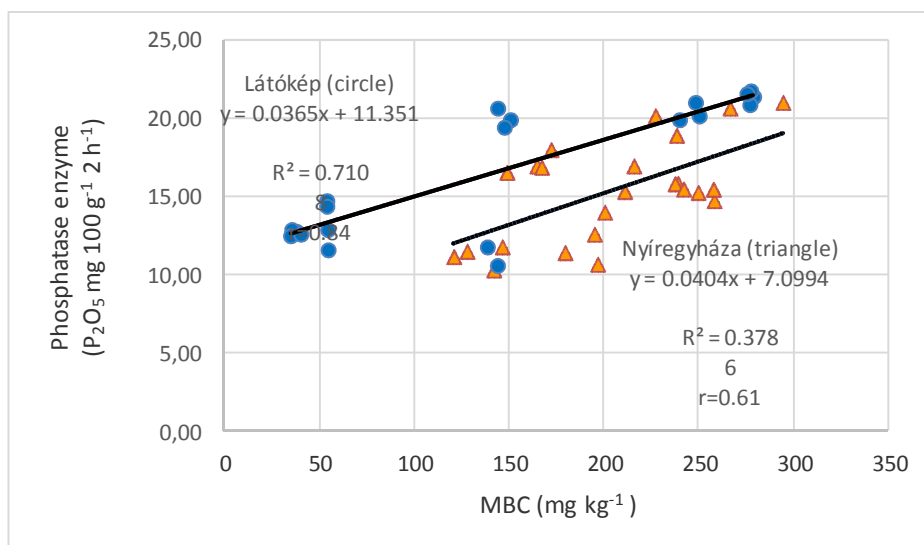


Figure 1

Correlation between MBC and phosphatase enzyme activity (Pearson's correlations, Nyíregyháza n=24; Debrecen – Látókép n=20)

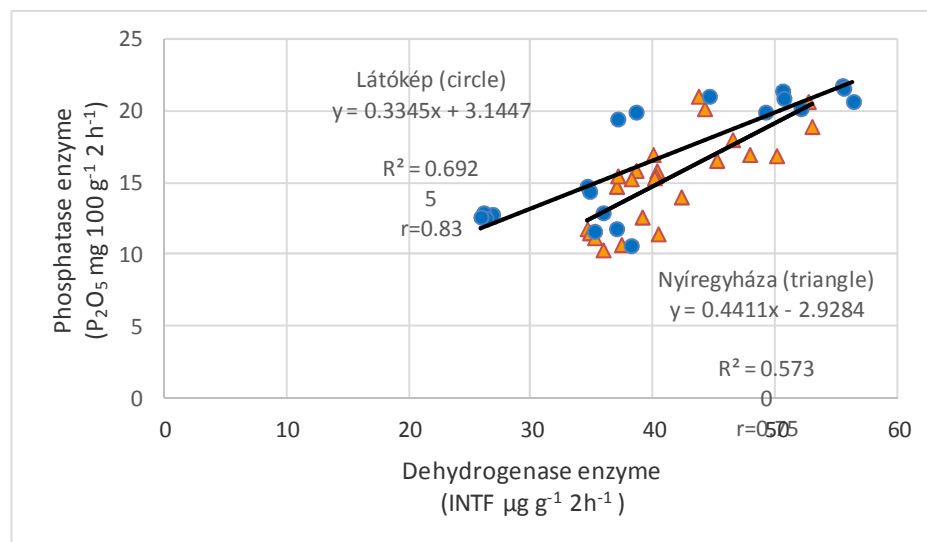


Figure 2

Correlation between the dehydrogenase and phosphatase enzyme activities (Pearson's correlations, Nyíregyháza n=24; Debrecen – Látókép n=20)

PIOTROWSKA-DŁUGOSZ and WILCZEWSKI (2015) found a similar phenomenon: green harvestings (catch crop, pea, *Pisum sativum* L.) increased MBC and the activity of soil enzymes, including saccharase, so these authors recommended greening for the stimulation of microbial activity. A similar conclusion was drawn by BOLTON et al. (1985) and LOSAKOV et al. (1986).

Comparison between organic and intensive farming conditions

When assessing the effects of organic and intensive farming on soil microbiological processes, the values of MBC, phosphatase and dehydrogenase activity were found to be higher in organic farming than in intensive farming, while higher carbon dioxide production and saccharase activity were measured in intensive farming.

Organic manure plus inorganic fertilizer increased the microbial biomass carbon by 74-99% (LIU et al., 2013). STEVLIKOVA et al. (2002) also demonstrated that MBC values were higher in organic than in intensive farming.

LUKÁCSNÉ and ZSUPOSNÉ (2008) reported higher values of phosphatase and dehydrogenase activity, MBC and CO₂ production in organic farming than in traditional farming systems. As in the present work, the saccharase enzyme activity proved to be higher in the traditional farming system.

BALEZENTIENE and KLIMAS (2009) found that nitrogen application stimulated the activity of saccharase, but the lowest values were measured with inorganic fertilization, suggesting that saccharase (and urease) activity is a bioactive property, usually positively correlated with soil nutrient content. In their view, the activity of

the two enzymes is a bio-indicator of soil fertility, responding well to the type of manure used and the system of nutrition management.

In summary, it can be concluded that straw, green manure and farmyard manure had a potent effect on the soil microbiological characteristics studied. Organic farming also contributed significantly to the increased activation of soil microbiological processes.

Conclusions

As a result of alternative nutrient supplies (A), the soil organic carbon content increased in all the treatments compared to the control. This increase was significant in the AF-M (inorganic fertilizer plus farmyard manure) and AGM-F (green manure plus inorganic fertilizer) treatments. The higher organic matter contents resulting from the treatments contributed to the significant enhancement of soil microbial parameters (MBC, saccharase, dehydrogenase and phosphatase enzyme activities).

The organic (OF) and intensive (I) cultivation treatments in the long-term experiment on calcareous chernozem soil (Debrecen-Látókép) had shorter life periods, only existing since 2000 and 1983, respectively.

Comparing the organic and intensive farming systems, it can be concluded that the carbon dioxide production and saccharase activity of organic plots (OF) were significantly lower than on intensive plots (I). In contrast, the microbial biomass carbon and the phosphatase and dehydrogenase activity were significantly higher in organic farm management systems (OF) than in the intensive farming system (I). These results are in harmony with data from the literature and also highlighted the need for sustainable production technologies.

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